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Heat Stress–Mediated Plasticity in a Locust Looming-Sensitive Visual Interneuron

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Money, Tomas G. A., Michael L. Anstey, and R. Meldrum Robertson. Heat stress–mediated plasticity in a locust looming-sensitive visual interneuron. *J Neurophysiol* 93: 1908–1919, 2005. First published November 24, 2004; doi:10.1152/jn.00908.2004. Neural circuits are strongly affected by temperature and failure ensues at extremes. However, detrimental effects of high temperature on neural pathways can be mitigated by prior exposure to high, but sublethal temperatures (heat shock). Using the migratory locust, *Locusta migratoria*, we investigated the effects of heat shock on the thermosensitivity of a visual interneuron [the descending contralateral movement detector (DCMD)]. Activity in the DCMD was elicited using a looming stimulus and the response was recorded from the axon using intracellular and extracellular methods. The thoracic region was perfused with temperature-controlled saline and measurements were taken at 5° intervals starting at 25°C. Activity in DCMD was decreased in control animals with increased temperature, whereas heat-shocked animals had a potentiated response such that the peak firing frequency was increased. Significant differences were also found in the thermosensitivity of the action potential properties between control and heat-shocked animals. Heat shock also had a potentiating effect on the amplitude of the afterdepolarization. The concurrent increase in peak firing frequency and maintenance of action potential properties after heat shock could enhance the reliability with which DCMD initiates visually guided behaviors at high temperature.

INTRODUCTION

Variations in temperature have profound effects on neural function and behavior of ectothermic organisms. Whereas physiological mechanisms exist that compensate for mild variations in ambient temperature (Heinrich 1993; Prange 1996; Prosser and Nelson 1981; Uvarov 1966; Willmer 1982), exposure to extreme temperature leads to dysfunction of the nervous system. Disruptions in signaling occur through direct effects of high temperature on the properties of proteins (Janssen 1992) and membranes (Hazel 1995; Hochachka and Somero 2002; Seddon 1990) of individual neurons. The thermosensitivity of action potentials and synaptic events result from temperature-dependent changes in the kinetics of ion channels (Janssen 1992; Montgomery and McDonald 1990). The effects of temperature set limits on the generation of action potentials, with conduction failures ensuing at the extremes of the range (Wu et al. 2001). Despite these constraints, many ectothermic animals are able to thrive under extreme as well as variable temperatures.

Signaling between neurons can be modified by experience with adaptive behavioral consequences. Prior exposure to a short-term, high but sublethal temperature (heat shock) has been shown to extend the operating range of neural processes when animals are exposed to a subsequent and otherwise lethal temperature (Marcuccilli and Miller 1994; Moseley 1993; Robertson et al. 1996). In locusts, heat shock extends the operating range of neural circuits (Dawson-Scully and Robertson 1998; Gray and Robertson 1998; Wu et al. 2001) and induces behavioral thermotolerance of flight rhythms (Robertson et al. 1996) and escape jumping (Barclay and Robertson 2000). Such plasticity has now been demonstrated for multiple components critical to signaling, including central synapses (Dawson-Scully and Robertson 1998), neuromuscular junctions (Barclay and Robertson 2000), and action potentials (Wu et al. 2001).

Plasticity in the shape of action potentials can permit animals to cope with a changing thermal environment (Rosenthal and Bezanilla 2002), and may thereby be an important mechanism for maintaining function under heat stress. Decreased amplitude and duration of action potentials at high temperature are thought to contribute to conduction failures (Lüscher et al. 1983; Stoney 1990; Westerfield et al. 1978). With respect to locust action potentials, increased temperature not only decreases amplitude and duration, but also hyperpolarizes membrane potentials and reduces input resistance (Abrams and Pearson 1982; Burrows 1989; Heitler et al. 1977; Simmons 1990; Wu et al. 2001; Xu and Robertson 1994). A variety of changes in action potential properties could improve conduction reliability along an axon, including spike broadening, maintenance of amplitude, or decreased refractory period. How heat shock affects the thermosensitivity of action potentials in actively conducting axon is unknown.

The locust descending contralateral movement detector (DCMD) is a visual interneuron sensitive to the looming approach of objects and is believed to be involved in triggering escape behaviors (Gabbiani et al. 1999, 2002; Gray et al. 2001; Rind and Simmons 1992). Features of an approaching object are computed in the brain by the lobula giant movement detector (LGMD; Gabbiani et al. 1999), and the information is passed to DCMD by a 1:1 chemical synapse (Rind 1984). DCMD acts as a relay of both low- and high-frequency spiking activity from LGMD in the brain to thoracic motor centers in the thoracic ganglia, making excitatory connections with neurons involved in both the jump (Burrows and Rowell 1973) and flight (Simmons 1980) motor patterns.

Escape jumping and flight rhythms are both modified by heat shock in ways that can be viewed as adaptive for the animal

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(Barclay and Robertson 2000; Robertson et al. 1996). Thermotolerance of behavior may be manifested by heat shock–induced changes in individual neuronal elements (Barclay and Robertson 2000; Klose et al. 2004; Wu et al. 2001). Thermotolerance in the propensity to execute an escape jump after heat shock implies that higher-order sensory processes may also be protected (Barclay and Robertson 2000).

The importance of faithful transmission of object approach information at high temperature makes the locust DCMD an excellent model for studying mechanisms of thermotolerance in the axon. In the present study, we examined how heat shock affects the thermosensitivity of action potentials in the DCMD axon during a ramped increase in thoracic temperature. We show that animals exposed to a heat shock have altered DCMD activity, such that the peak firing frequency is increased. We also show that the properties of action potentials in the DCMD axon are more thermotolerant after heat shock. The findings indicate that there is plasticity in the DCMD response to temperature. These heat shock–induced changes can be viewed as adaptive by promoting thermotolerance in the animal.

METHODS

Animals

Experiments were performed on adult male locusts, *Locusta migratoria*, taken 3 to 4 wk after their final molt from a crowded colony maintained in the Department of Biology at Queen's University. The photoperiod of the colony was set to a 12 h:12 h light:dark cycle. Cage temperature was 25 ± 1°C during light hours and 21 ± 1°C during dark hours, with a constant humidity of 23 ± 1%. Locusts were assigned to either a control or a heat-shocked treatment group. Heat-shocked animals were placed in a 2-L ventilated plastic container in a humid incubator (45°C) for 3 h. After the heat shock, animals were left to recover at room temperature (22 ± 1°C) for a minimum of 1 h and a maximum of 5 h. Control animals were placed in similar containers at room temperature for 4–8 h before an experiment.

Preparation

A semi-intact preparation (Robertson and Pearson 1982) was used to expose the thoracic nervous system (Fig. 1*A*). An incision was made along the dorsal midline and the animal was pinned to expose the thoracic ganglia. After removal of the overlying tissue, the meso and metathoracic ganglia were mounted on a metal plate. Nerves 3, 4, and 5 of each ganglion were bilaterally cut to minimize movement of the preparation.

A Peri-Star peristaltic pump (World Precision Instruments, Sarasota, FL) was used to superfuse the preparation with standard locust saline containing (mM): 147 NaCl, 10 KCl, 4 $CaCl_2$, 3 NaOH, and 10 HEPES buffer (pH 7.2). The saline dripped into the anterior portion of the thoracic cavity, flowed posteriorly over the thoracic nervous system, and exited through a cut in the anterior portion of the abdomen. Temperature of the preparation was controlled by passing current through a coil of Nichrome wire wrapped around the glass pipette used to direct the saline into the thoracic cavity. A thermocouple (BAT-12; Physitemp Instruments, Clifton, NJ), placed about 0.5 mm away from the mesothoracic ganglion, was used to monitor temperature.

Temperature was raised by 5°C/min from 25°C with one measurement taken at each temperature or until no action potentials were generated in response to the stimulus (failure). For a subset of animals ($n = 8$), the temperature of the thorax and brain was measured simultaneously during a temperature ramp. At room temperature, there was no difference observed between the temperature of the head and the temperature of the thorax. As the temperature of the thorax was raised to 45°C, the temperature of the head increased more slowly, reaching a maximum of only 29°C.

Recordings

Action potentials were recorded intracellularly from the DCMD. The axon of this interneuron was penetrated just posterior to the mesothoracic ganglion in the mesothoracic to metathoracic connective (Fig. 1, *A* and *B*). Glass microelectrodes containing 1 M KAc (20–60 MΩ) were used for all intracellular recordings except those used to measure input resistance, in which 3 M KCl (10 MΩ) electrodes were used. Intracellular recordings were amplified using a Neuroprobe Model 1600 amplifier (A-M Systems, Everett, WA). Effort was made

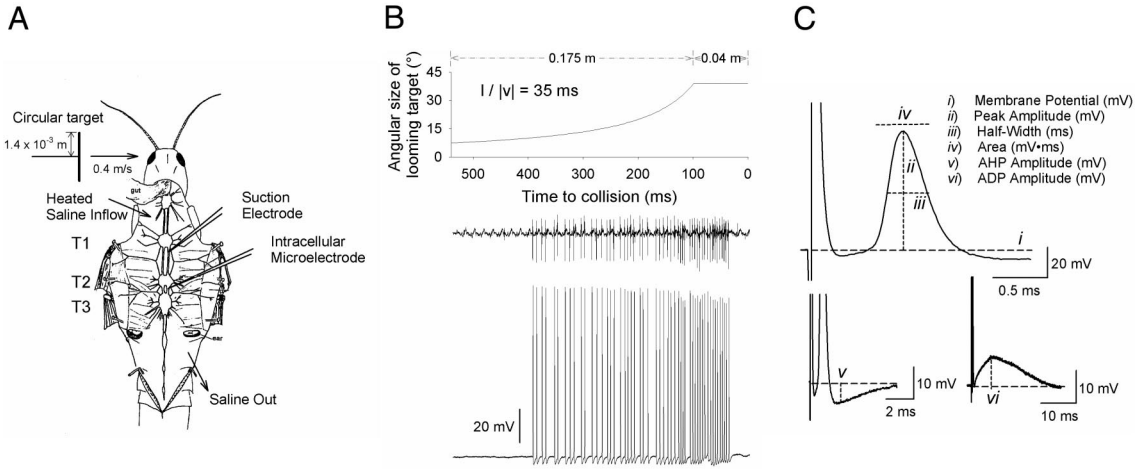

FIG. 1. Experimental preparation. Activity of the descending contralateral movement detector (DCMD) in response to a looming stimulus was recorded in *L. migratoria* using electrophysiological techniques. *A*: extracellular suction electrode was placed on the right connective at a position 0.5–1 mm anterior of the mesothoracic ganglion and an intracellular microelectrode was inserted in the right axon just posterior to the mesothoracic ganglion. Target was located 21.5 cm away from the eye and subtended an angle of 7°. Target approached on a direct collision course at a velocity of 0.4 m/s. *B*, *top panel*: angular size of the target during approach. Corresponding DCMD response measured in simultaneous extracellular and intracellular recordings are shown below. *C*: action potentials were stimulated in DCMD through a suction electrode. Membrane and action potential properties were compared between control and heat-shocked (3 h, 45°C) animals. Resting membrane potential is indicated by the long-dashed line (*i*). Measurements included peak amplitude (*ii*), half-width (*iii*), and area (*iv*). Afterpotential was characterized by the amplitude of its afterhyperpolarization potential (AHP; *v*) and afterdepolarization potential (ADP; *vi*).

to penetrate DCMD in the same location for all animals used in these experiments. The DCMD neuron was easily identifiable when penetrated because movement within the locust's visual field contralateral to the recording site resulted in robust spiking. Successful DCMD penetrations were given 5–10 min to stabilize before beginning the experiment.

For experiments examining the response of DCMD to a looming target, extracellular activity in the DCMD was recorded in addition to intracellular recordings. A suction electrode was placed on the dorsomedial surface of the right connective at a position 1 ± 0.5 mm anterior of the mesothoracic ganglion. The signal was amplified using a Model P15 Preamplifier (Grass Instruments, West Warwick, RI). Activity in the DCMD could be reliably identified by the size of the spikes relative to other activity and background noise level, and was confirmed by simultaneous intracellular recordings (Fig. 1, *A* and *B*). Extracellular and intracellular signals were digitized using a Digidata 1322A (Axon Instruments, Union City, CA) and recorded to computer using Axoscope 9.0.

For experiments in which action potential properties were examined, a stimulating suction electrode was used. The extracellular stimulating electrode was connected to a Grass Model S48 Stimulator (Grass Instruments). Stimulation with voltages just suprathreshold resulted in a faithful 1:1 stimulation to spike ratio. The presence of the suction electrode did not affect the visual sensitivity of DCMD.

Looming stimulus

The looming stimulus was generated by mounting a circular target (radius of 1.4 cm) onto a mechanical track (*x–y* plotter). Target approach was driven by a square voltage pulse delivered by a Grass Model SD9 Stimulator (Grass Instruments). The square voltage pulse was recorded to signal the start of the target approach. The circular target was white with a 2-mm black border and a 2-mm black crosshair through its center. The target traveled at a velocity of 0.4 m/s on a direct collision trajectory to the locust's left eye at 90° to the body axis (Fig. 1*A*).

As an object approaches, it subtends across an increasingly greater angle of the visual field. The dynamic appearance of a looming object's approach is dependent on the ratio of the object's half-size (l) to the object's approach velocity (v). The angular size of the object on the retina at time t can be expressed as angle $t = 2 \tan^{-1} [l/v \times (t)]$. The approach parameters used in these experiments result in an l/v value of 35 ms. Before the approach, the target was located 21.5 cm away from the eye, and subtended an angle of 7°. Target movement stopped 100 ms before projected collision with a final distance from the eye of 4 cm at an angular extent of 39° (Fig. 1*B*).

Analysis of response to looming stimulus

Responses to the looming approach of a target were recorded from the axon of DCMD. The number of action potentials evoked per looming stimulus, and their timing relative to projected collision, were analyzed at 25, 30, 35, 40, and 45°C during the temperature ramp. Only animals that responded at 35°C or higher were used. Initial sample sizes were 23 control animals and 23 heat-shocked animals ($n_C = 23$; $n_{HS} = 23$). There was a decrease in the sample size at 40°C ($n_C = 17$; $n_{HS} = 20$), and a further decrease at 45°C ($n_C = 12$; $n_{HS} = 12$) because some animals failed to respond at higher temperatures.

The DCMD response was measured from the start of target movement until projected collision. In the absence of visual stimulation, spontaneous firing rates in DCMD were very low (<0.1 Hz; data not shown). As a result, spontaneous action potentials are not likely to have participated significantly in any of the spike occurrence or latency data. Conduction velocity was measured by dividing the distance between the extracellular and intracellular electrodes by the time required for an action potential to conduct between them.

The time difference between adjacent spikes was used to calculate an instantaneous frequency for all action potentials. To examine differences in the DCMD firing pattern, instantaneous frequency data for each action potential were averaged into time bins of 20 ms for each animal at each temperature. Over the total target approach time of 537.5 ms, this yielded 27 time bins. The first time bin was designated as a 17.5-ms time bin.

Analysis of action potential properties

In a separate series of experiments, action potentials recorded from the axon of DCMD were analyzed at 25, 30, 35, and 40°C during the temperature ramp. Sample sizes were 14 control animals and 12 heat-shocked animals. There was a decrease in the sample size at 40°C ($n_C = 12$; $n_{HS} = 11$) as a result of conduction failure or loss of the intracellular penetration.

Only DCMD recordings with resting membrane potentials (V_m) more negative than −50 mV and action potential amplitudes >80 mV at 25°C were used. Resting membrane potential was measured directly from the digitized intracellular record. Input resistance at the recording site was calculated from the intracellular trace using the measured response of the membrane to a known current step protocol. Steady-state voltage responses were used to construct voltage versus current (*V/I*) plots, from which the slopes were used to estimate input resistance. Measurements of the action potential included peak amplitude, half-width, and area (Fig. 1*C*). Measurements of the afterpotential were separated into 2 components: afterhyperpolarization amplitude (AHP) and afterdepolarization amplitude (ADP; Fig. 1*C*). AHP was measured as the most negative point after the decay phase of the action potential. ADP amplitude was measured as the peak level of depolarization after the afterhyperpolarization. In all recordings, AHP minima were clearly distinguishable. All amplitude measurements were made relative to the resting membrane potential immediately preceding the action potential at each temperature.

Statistics

Statistical significance of the effects of heat shock on the thermosensitivity of DCMD responses was assessed by ANOVA followed by a *t*-test ($P < 0.05$) between control and heat-shocked treatments at specific temperature conditions. ANOVA tests are reported as $F_{(a,b)} = X$, where a is the degrees of freedom and b is the residual sum of squares. To test the effect of temperature within a treatment (control or heat shock) a one-way ANOVA test was used. Nonparametric ANOVA tests (Kruskal–Wallis ANOVA on ranks) were used in instances where normality and equal variance criteria of the data were not met. Two-way ANOVA comparisons were used to examine thermosensitivity differences between control and heat-shocked treatments. Differences in the firing frequency of DCMD responses during the looming approach of the target were assessed using a 2-way repeated-measures ANOVA (RM-ANOVA). A repeated-measures test was used because spike frequency data were available for each 20-ms bin of target approach for an individual at each temperature. Differences in the occurrence frequency of parameters, such as the presence or absence of an event at a specific temperature, were analyzed by using the nonparametric Fisher's Exact Test ($P < 0.05$). Statistical tests were performed using SigmaStat v3.0 software. Data are reported as means ± SE in the text and figures. Figures and SE bars were generated using SigmaPlot 8.0.

RESULTS

Heat shock and the response to a looming object

The response in DCMD to a looming stimulus was compared over different temperatures between control and heat-

shocked animals. As temperature increased, resting membrane potential was hyperpolarized and action potential amplitude decreased in both control and heat-shocked animals (Fig. 2). Increasing the temperature of the thorax resulted in a decrease in the number of spikes being recorded in control animals [one-way ANOVA; $F_{(4,93)} = 7.8$, $P < 0.001$; Fig. 3*A*]. Heat-shocked animals showed no change in spike count across different temperatures [Kruskal–Wallis one-way ANOVA on ranks; $H_{(4)} = 1.141$, $P = 0.889$]. Spike count between control and heat-shocked animals was significantly different [2-way ANOVA; $F_{(1,189)} = 45.2$, $P < 0.001$]. Significant differences were revealed at 30, 35, 40, and 45°C (*t*-test, $P < 0.05$).

The time of the first spike relative to collision was compared between control and heat-shocked animals with increasing temperature. The response started later in control animals with each successive increase in temperature [Kruskal–Wallis one-way ANOVA on ranks; $H_{(4)} = 10.6$, $P = 0.03$]. Timing of the first spike was similar at all temperatures in heat-shocked animals [one-way ANOVA; $F_{(4,187)} = 1.6$, $P = 0.189$]. Time of first spike relative to collision was significantly different between control and heat-shocked animals [2-way ANOVA; $F_{(1,187)} = 26.5$, $P < 0.001$; Fig. 3*B*]. Significant differences were revealed at 30, 35, 40, and 45°C (*t*-test, $P < 0.05$).

Conduction velocity was measured over a short stretch of axon (1 mm). Increased temperature significantly increased the conduction velocity in the axon [2-way ANOVA, $F_{(3,112)} = 38.6$, $P < 0.001$; Fig. 3*C*], although control and heat-shocked treatments were indistinguishable [2-way ANOVA, $F_{(1,112)} = 2.0$, $P = 0.160$]. Although conduction velocity in the axon was strongly affected by temperature, there was no change in its thermosensitivity after heat shock.

Effect of heat shock on firing frequency

The firing frequency in both control and heat-shocked animals increased continuously throughout target approach, reaching a peak before decreasing rapidly by projected collision (Fig. 4). The peak of the response occurred after the target had come to rest at an angular size of 39° in both control and heat-shocked animals. At 25°C, the peak of the response in control animals averaged from 80 to 40 ms before collision, and from 60 to 40 ms after heat shock. The response to the looming stimulus in control and heat-shocked animals was indistinguishable at this temperature [2-way RM-ANOVA; $F_{(1,817)} = 1.8$, $P = 0.199$; Fig. 4].

Activity in DCMD elicited throughout target approach differs between 25 and 45°C in both control and heat-shocked animals. The firing frequency during the early phase of the response in both control and heat-shocked animals was lower at 45 than that at 25°C. The decreased rate was more prolonged in control than in heat-shocked animals. The firing frequency at 45°C was increased around the peak relative to the response at 25°C in heat-shocked, but not control animals. In heat-shocked animals at 45°C, there was a peak at 200–180 ms to collision (at an angular size of 22°), which preceded the main peak at 80–60 ms to collision. Throughout the response, the frequency of DCMD activity was significantly higher at 45°C in heat-shocked animals compared with control animals [2-way RM-ANOVA; $F_{(1,430)} = 15.3$, $P < 0.001$; $n_C = 12$, $n_{HS} = 12$; Fig. 4].

The increase in firing frequency found in heat-shocked animals at high temperature was not the result of a sustained increase in firing rate. Rather, the increase in firing frequency at 45°C in heat-shocked animals arose from short episodes of spikes firing at very high frequencies (Fig. 5*A*). Heat-shocked

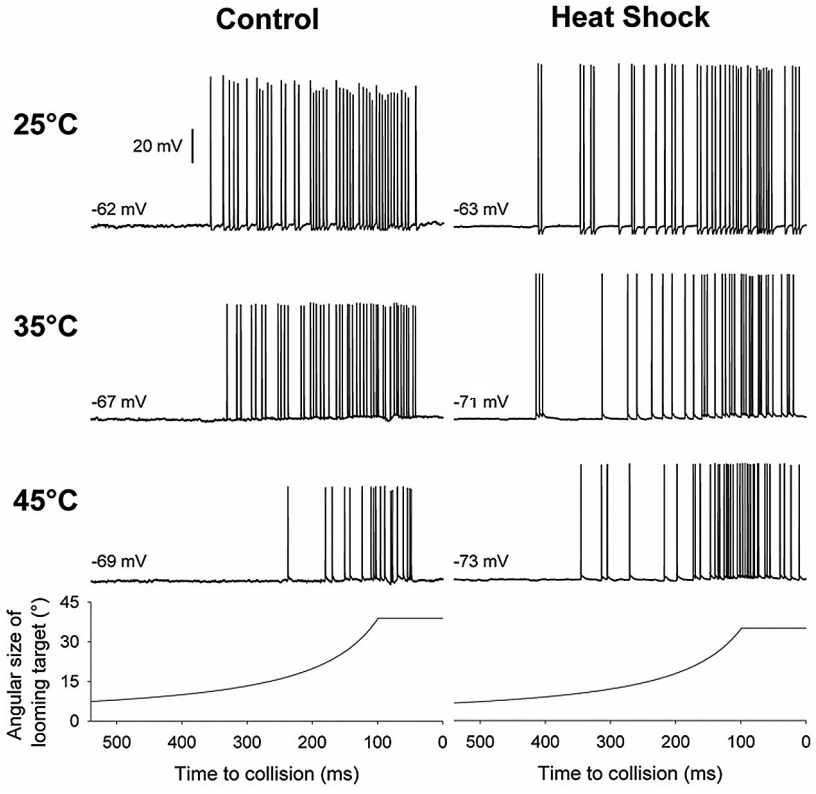

FIG. 2. Intracellular recordings from DCMD in response to a looming target. At 25°C both heat-shocked and control locusts showed a characteristic response to the looming target. Spike frequency increased, after a delay, to a peak response after the target came to a stop (100 ms before projected collision). Resting membrane potential was hyperpolarized and action potential amplitude decreased with increasing temperature. Heat-shocked locusts were able to maintain high-frequency firing at elevated temperatures whereas control locusts were not.

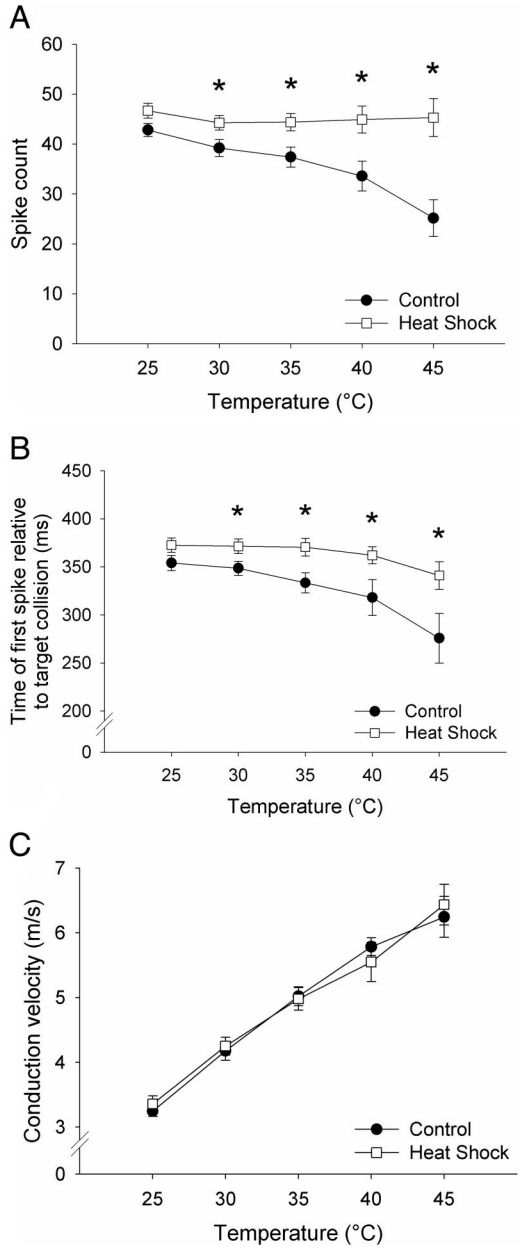

FIG. 3. Temperature-dependent changes in the DCMD response to the looming target. Data are presented as means ± SE. Significant differences as assessed by a *t*-test ($P < 0.05$) are denoted with asterisks. *A*: spike count was affected by temperature in control but not heat-shocked animals. As temperature increased from 25 to 45°C, the number of spikes produced in response to a looming stimulus decreased in control (filled circles) animals. Heat-shocked (open squares) animals maintained their spike count at all temperatures. Spike count was significantly different between control and heat-shocked animals at all temperatures except 25°C. *B*: there was a temperature-dependent change in the time of the first spike relative to collision. Start of the DCMD response was significantly delayed in control compared with heat-shocked animals at all temperatures except 25°C. *C*: conduction velocity was not affected by heat shock. Conduction velocity of action potentials in the DCMD axon increased with temperature, but was not affected by prior heat shock.

animals had bursts that reached higher instantaneous frequencies than control at all temperatures except 25°C (Fig. 5*B*). At high temperature, the responses in heat-shocked animals had spikes with instantaneous frequencies often >600 Hz. Spikes from control animals were not found to have instantaneous frequencies of this rate at any temperature. The highest instantaneous frequency was significantly greater after heat shock compared with control [2-way ANOVA; $F_{(1,142)} = 26.5$, $P < 0.001$; Fig. 5*C*]. Significant differences were revealed at 30, 35, 40, and 45°C (*t*-test, $P < 0.05$).

Effect of heat shock on action potential properties

The properties of action potentials in the DCMD axon were measured in control and heat-shocked animals as the temperature of the saline was increased (Fig. 6, *A* and *B*). The resting membrane potential (V_m) hyperpolarized significantly with increasing temperature (Fig. 6*C*) in both control and heat-shocked preparations [2-way ANOVA; $F_{(3,93)} = 15.62$, $P < 0.001$]. At 25°C, the mean V_m for control and heat-shocked animals was −60.0 ± 1.2 and −62.6 ± 1.3 mV, respectively. At 40°C, the membrane potential hyperpolarized to −67.9 ± 1.1 mV in control and −72.5 ± 1.9 mV after heat shock. There was a significant difference between the membrane potential in control and heat-shocked animals across the temperatures examined [2-way ANOVA; $F_{(1,93)} = 16.35$; $P < 0.001$]. Resting membrane potential was significantly more hyperpolarized in heat-shocked animals at all temperatures except 25°C (*t*-test, $P < 0.05$).

The action potential amplitude decreased significantly with increasing temperature (Fig. 6*D*) in both control and heat-shocked preparations [2-way ANOVA; $F_{(3,89)} = 51.19$, $P < 0.001$]. At 25°C, the mean amplitude of action potentials in control and heat-shocked animals was 91.1 ± 1.9 and 93.6 ± 2.1 mV, respectively. At 40°C, the amplitude decreased to 65.0 ± 1.8 mV in control and 72.3 ± 2.7 mV after heat shock. There was a significant difference between the action potential amplitude in control and heat-shocked animals across the temperatures examined [2-way ANOVA; $F_{(1,89)} = 15.33$; $P < 0.001$]. Action potential amplitude was significantly greater in heat-shocked animals at all temperatures except 25°C (*t*-test, $P < 0.05$).

The action potential half-width decreased significantly with increasing temperature (Fig. 6*E*) in both control and heat-shocked preparations [2-way ANOVA; $F_{(3,93)} = 24.03$, $P < 0.001$]. At 25°C, the mean half-width for control and heat-shocked animals was 0.32 ± 0.02 and 0.33 ± 0.02 ms, respectively. At 40°C, the amplitude decreased to 0.16 ± 0.01 ms in control and 0.17 ± 0.01 ms after heat shock. There was no significant difference between the action potential half-width in control and heat-shocked animals across the temperatures examined [2-way ANOVA; $F_{(1,93)} = 0.185$, $P = 0.67$].

The action potential area decreased significantly with increasing temperature (Fig. 6*F*) in both control and heat-shocked preparations [2-way ANOVA; $F_{(3,91)} = 32.04$, $P < 0.001$]. At 25°C, the mean area for control and heat-shocked animals was 30.9 ± 1.5 and 33.2 ± 1.8 mV/ms, respectively. At 40°C, the amplitude decreased to 12.4 ± 0.5 mV/ms in control and 14.8 ± 1.8 mV/ms after heat shock. Although there was a significant difference between the action potential area in control and heat-shocked animals across the temperatures examined [2-way ANOVA; $F_{(1,91)} = 6.54$, $P < 0.012$], *t*-tests showed no significant difference at any of the temperatures examined.

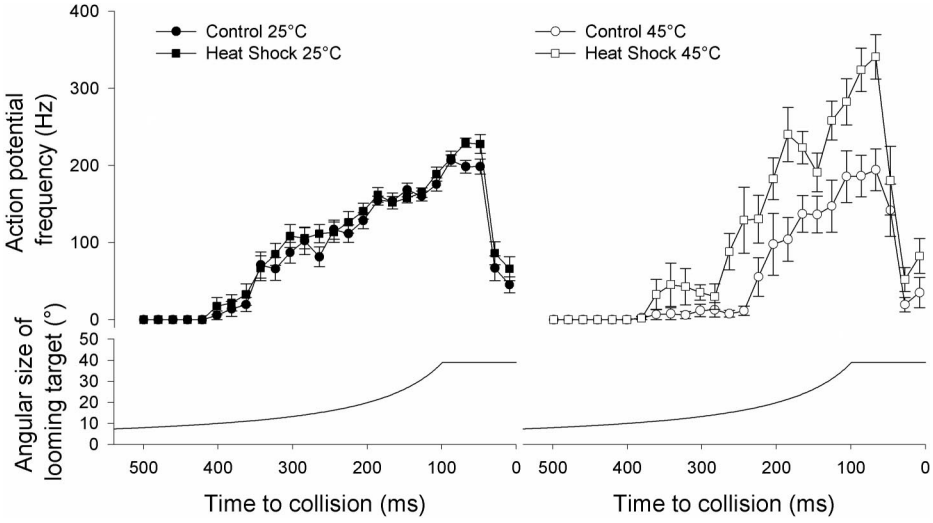

FIG. 4. Frequency of the DCMD response to a looming object was different in control and heat-shocked animals at high temperature. Burst patterns were compared by grouping the instantaneous frequency of spikes into 20-ms bins. DCMD response frequency in control (filled circles) and heat-shocked (filled squares) animals at 25°C was indistinguishable. Frequency increased with object approach. Maximal frequency (peak response) in control occurred between 80 and 40 ms to collision and between 60 and 40 ms after heat shock. Peak DCMD activity occurred after the target had stopped its approach in both control and heat-shocked animals. Action potential frequency decreased sharply after reaching its peak. At 45°C there was a reduction of early spikes in both control (open circles) and heat-shocked (open squares) animals, although more prolonged in control than heat shock. There was an increase in spikes late in the burst (around peak) in heat-shocked but not control animals at 45°C.

Effect of heat shock on the afterpotential

The afterpotential was examined by measuring the amplitude of the AHP and the ADP. These two events occurred with different time courses (Fig. 7*A*), and were clearly distinguishable. There was no significant difference in the mean amplitude of the AHP (*t*-test; $t = 1.52$; $P = 0.14$; df = 24) between control and heat-shocked animals at 25°C. An ADP was present at 25°C in 14% of control animals (2/14) and 42% of heat-shocked animals (5/12), but this difference was not statistically significant (Fisher's Exact Test; $P = 0.19$).

Increased temperature was found to affect the amplitude of both the AHP and the ADP. The afterpotential changed gradually from hyperpolarized to depolarized with temperature, resulting in an increased ADP and an AHP that became depolarized relative to the resting potential (Fig. 7*B*). At 35°C significantly more heat-shocked animals (92%) had an ADP than control animals (43%; Fisher's Exact Test; $P < 0.05$).

Heat-shocked animals exhibited action potentials with ADP amplitudes that were found to be significantly larger than control across the temperatures examined [Fig. 7*C*; 2-way ANOVA; $F_{(1,93)} = 25.32$; $P < 0.001$]. At 40°C the mean amplitude of the ADP increased to 2.2 ± 0.6 mV in control and 6.0 ± 1.2 mV after heat shock. ADP amplitude was significantly greater in heat-shocked animals at all temperatures except 25°C (*t*-test, $P < 0.05$). Amplitude differences in the ADP were not attributed solely to the increased incidence of ADPs in heat-shocked animals at high temperature. When considering only the animals that had a depolarized afterpotential at 40°C (Fig. 7*D*), the amplitude of the ADP in control animals was significantly lower (3.3 ± 0.5 mV; $n = 8$) compared with 6.6 ± 1.1 mV ($n = 10$) in heat-shocked animals ($t = 2.36$; $P < 0.05$, df = 16).

Effect of heat shock on input resistance

The input resistance of the DCMD axon was examined at 25 and 35°C by measuring the voltage response to known levels of injected current. In both control and heat-shocked animals the voltage response was linear (Fig. 8). There was no significant interaction between the steady-state voltage response to current injection and treatment condition (control or heat-shocked) at 25°C [2-way ANOVA; $F_{(10,87)} = 0.37$, $P = 0.96$]. The slope of a linear regression of the *V/I* data gave input resistance values of 2.3 MΩ for control and 2.0 MΩ for heat-shocked animals. The voltage response was lower at 35°C (Fig. 8). There was a significant interaction between the steady-state voltage response to current injection and treatment condition (control or heat-shocked) at 35°C [2-way ANOVA; $F_{(10,38)} = 10.3$, $P < 0.001$]. Input resistance of DCMD at 35°C was 0.78 MΩ in control and 0.21 MΩ after heat shock. These findings suggest that the temperature-dependent decrease in input resistance was more pronounced after heat shock than for control animals.

Increase in membrane excitability after heat shock

In response to a brief hyperpolarizing pulse, 64% (7 of 11) of heat-shocked animals demonstrated a postinhibitory rebound (PIR; Fig. 9*A*), at temperatures >40°C. The PIR activity was induced rapidly, reaching its maximum amplitude of 8.3 ± 1.2 mV within 5.1 ± 1.1 ms of initiation. These excitability events were observed less often in control animals (25%; 3 of 12), and never at lower temperature in either control or heat-shocked animals. In 3 instances after heat shock, the postinhibitory rebound was sufficient to generate a burst of spikes (Fig. 9*B*). Further, a spike train was triggered by a single stimulation of the DCMD axon. The action potentials in the burst, initiated both as a postinhibitory rebound as well as after a stimulated action potential, had characteristics distinct from the electrically stimulated action potentials, with decreased amplitude and more slowly activating rising phases (Fig. 9*C*).

DISCUSSION

The descending contralateral movement detector (DCMD) is a visual interneuron that relays information about the looming approach of objects from the brain to the motor centers in the thorax. Activity in DCMD is believed to be involved in the initiation of escape behavior in the locust (Gabbiani et al. 1999, 2002; Rind and Simmons 1992) as well as steering avoidance during flight (Gray et al. 2001). We examined how the DCMD's response to a looming stimulus was affected by a ramped increase in thoracic temperature in control and heat-shocked locusts. Activity in DCMD was

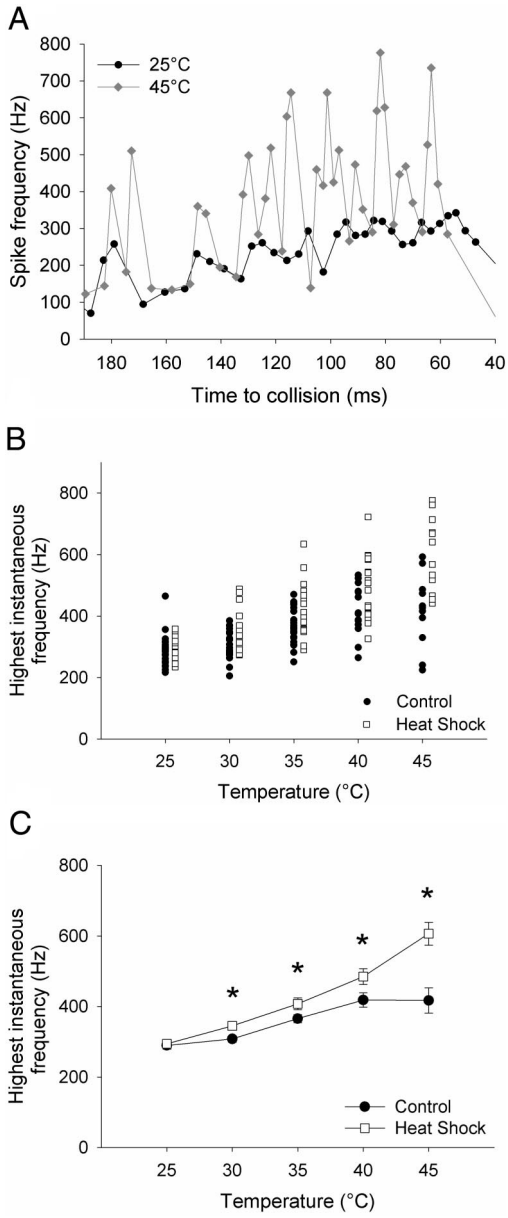

FIG. 5. Highest instantaneous frequency was increased at high temperature after heat shock. *A*: data from a single representative animal showing that the increase in mean firing frequency at 45°C (light diamonds) in heat-shocked animals arose from short episodes of spikes firing at very high frequencies. High-frequency doublets or triplets were followed by periods where the spike frequency was similar to that at 25°C (dark circles). *B*: heat-shocked animals had bursts that reached higher instantaneous frequencies than control at all temperatures except 25°C. At 45°C many heat-shocked animals fired spikes with instantaneous frequencies that exceed 600 Hz. *C*: instantaneous frequency in DCMD of heat-shocked animals was significantly greater at all temperatures except 25°C. Data are presented as means ± SE. Significant differences as assessed by a *t*-test ($P < 0.05$) are denoted with asterisks.

decreased in control animals with increased temperature, whereas heat-shocked animals had a potentiated response such that the peak firing frequency was increased. In addition, significant differences were found in the thermosensitivity of DCMD action potential properties between control and heat-shocked animals. Heat shock also had a potentiating effect on the ADP, which occurred more frequently and with greater amplitude than in control animals.

Plasticity in the DCMD response to temperature

Increased temperature lowered the DCMD mean firing frequency early in the response in both control and heat-shocked animals. The reduction in the number of spikes, however, was less severe in heat shock than in control. Starting the DCMD response later, and with fewer spikes may impair the behavioral outcome by decreasing the time available to signal an approaching object.

There was no difference in the conduction velocity between control and heat-shocked animals, indicating that the observed latency difference was not the result of a slowing of action potential conduction down the axon of control animals. Heat shock has been shown to reduce the thermosensitivity of conduction velocity in the locust forewing stretch receptor axon (fSR; Gray and Robertson 1998). It is not clear why heat shock would differently affect conduction velocity of DCMD and fSR axon. Conduction of action potentials in DCMD was slightly faster than has been reported for fSR (Gray and Robertson 1998). Decreased thermosensitivity in the fSR contributes to an overall decrease in the thermosensitivity of the locust flight rhythm after heat shock, which is thought to help maintain the flight circuit within an optimal operating range (Gray and Robertson 1998; Robertson et al. 1996). Because DCMD is thought to function to signal impending collision (Rind and Simmons 1992; Schlotterer 1977), perhaps the importance of rapid conduction in DCMD precludes a reduced thermosensitivity as is found in fSR.

Although still contentious (Rind and Santer 2003), peak firing frequency in DCMD is thought to signal an object's approach because it consistently occurs with a fixed delay after the object subtends a set angle on the locust eye (Gabbiani et al. 1999). In this study, the peak firing rate in both control and heat-shocked animals occurred after the target had come to a stop. We cannot discount the possibility that the reduction of firing was a response to the halted approach, making it difficult to address how and what object cues (angular size, edge acceleration) are being processed. However, it was not the purpose of our study to examine the precise processing of object approach by LGMD/DCMD. Rather, we have taken a comparative approach to examine how the pattern differs after heat shock in its response to temperature.

Although there was little change in the time of the peak response between temperatures, the firing frequency of heat-shocked animals was significantly higher at 45°C when compared with the response at 25°C. It appears that the maintenance of spike count observed at high temperature in heat-shocked animals arises from a potentiation of number of spikes during the peak firing time. In heat-shocked animals, we also saw a small peak 25 ms before the target has reached an angle of subtense of 25°C. This early peak was absent in control animals. It is an interesting possibility that the potentiation represents a compensatory response to the early loss of spikes in the train.

The increased firing frequency shown in heat-shocked animals was not uniform throughout the response, but rather occurred as short episodes of very high frequency activity. Instantaneous frequencies during these episodes surpassed 600 Hz in a number of animals, a rate not seen at room temperature. It is unknown how DCMD was modified by heat shock to bring about this change in the response pattern at high temperature.

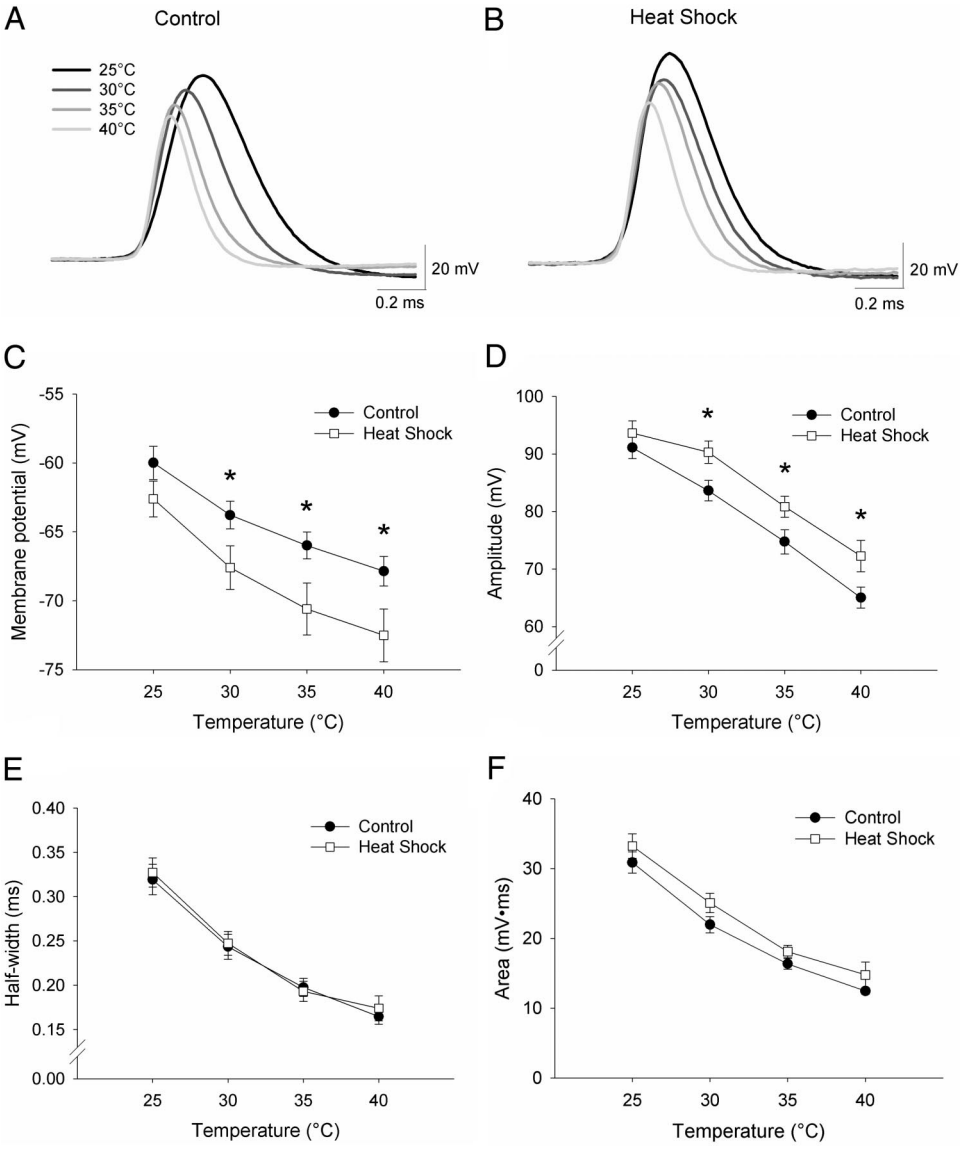

FIG. 6. Effect of temperature on action potential properties. Action potentials were compared between control (filled circles; $n_C = 14$) and heat-shocked (open squares; $n_{HS} = 12$) animals at each of 4 temperatures (25, 30, 35, 40°C). Data are expressed as means ± SE. Significant differences as assessed by a *t*-test ($P < 0.05$) are denoted with asterisks. Action potentials in both control (*A*) and heat-shocked (*B*) animals were affected by temperature. *C*: membrane potential was hyperpolarized with increasing temperature in both control and heat shock. Heat-shocked animals were significantly more hyperpolarized than control animals at all temperatures except 25°C. *D*: amplitude was decreased with increasing temperature in both control and heat shock. Heat-shocked animals had action potentials with significantly greater amplitude than control animals at all temperatures except 25°C. *E*: half-width was reduced as temperature increased in both control and heat shock, with no difference between control and heat-shocked animals at all temperatures. *F*: area decreased with increasing temperature in both control and heat shock. Although there was a significant difference between control and heat-shocked animals across all temperatures [2-way ANOVA; $F_{(1,91)} = 6.54$, $P < 0.012$], there was no difference between control and heat shock at any individual temperature.

There is growing evidence that intrinsic excitability properties of neurons show plasticity (Destexhe and Marder 2004), and that target activity levels can be altered through experience-driven mechanisms (Zhang and Linden 2003). The spike transmission ratio between neurons is subject to short-term plasticity during sustained activity (Blitz et al. 2004). The same presynaptic stimulus can elicit responses with failures, faithful transmission, or can result in multiple action potentials per presynaptic action potential (Blitz and Regehr 2003). The LGMD/DCMD synapse is thought to have 1:1 transmission fidelity (Rind 1984), but it is unknown whther this holds at the high temperatures used in our experiments. Plasticity in the transfer ratio to DCMD would explain the altered activity observed at high temperature after heat shock (see Fig. 5 herein).

Despite the evidence that the peak firing frequency in DCMD reliably indicates angular threshold of a looming object (Gabbiani et al. 1999, 2002; Matheson et al. 2004), it has yet to be conclusively shown how DCMD triggers behavioral responses on a time scale appropriate to be useful in an escape maneuver (Burrows 1996; Gabbiani et al. 2004). The timing of the initiation of steering-avoidance behavior in the locust relative to collision (200 ms at an l/v of 20; Matheson et al. 2004), or angular threshold (10° with a delay; Robertson and Johnson 1993), suggests that the time of the peak DCMD response in our results occurs too late to be involved in the initiation of such behaviors. However, Hatsopoulos et al. (1995) showed that peak DCMD activity correlates with the rapid initiation of a jump (Gabbiani et al. 1999).

The DCMD firing frequency in heat-shocked animals is greater than control for much of the target approach. It is possible that the divergent responses generate different behavioral outcomes in these animals. It has been demonstrated that the firing rate also varies linearly with l/v (Gabbiani et al. 2002, 2004), suggesting that a threshold firing frequency could be used to indicate an object's angular size. A similar DCMD threshold mechanism has been suggested by others for the control of jumping (Rind and Santer 2003; Rind and Simmons 1992). If this hypothesis is accurate, the heat shock–induced change we describe in the early part of the response would have greater impact in adaptively modifying behavior than the potentiation centered around the peak.

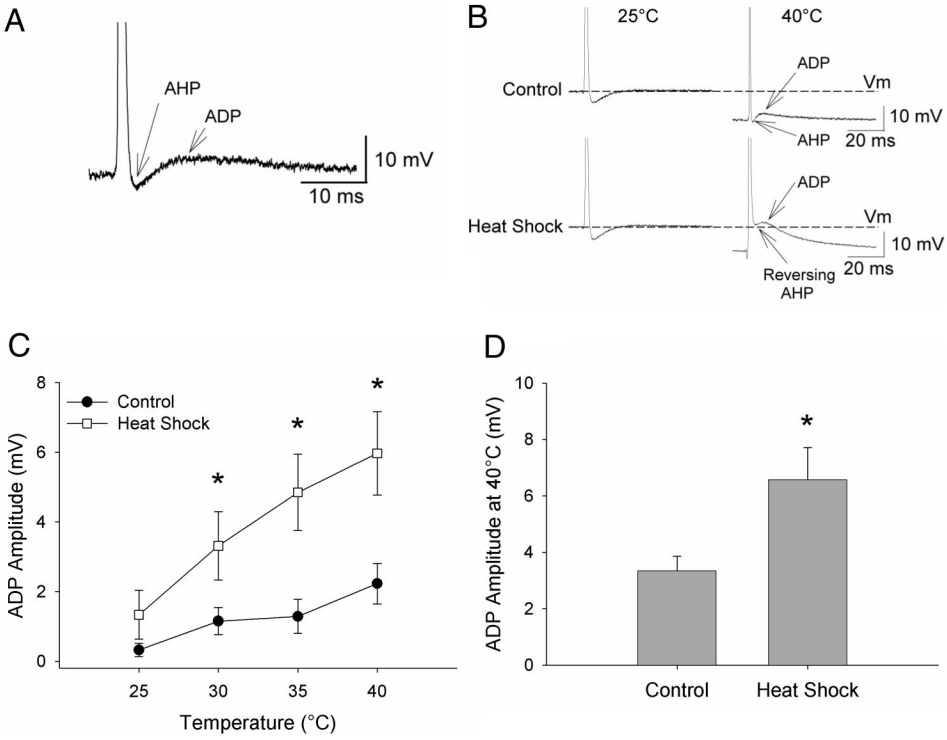

FIG. 7. Heat shock increased the depolarizing component of the afterpotential at high temperature. *A*: action potential at 25°C with an AHP and an ADP. Action potential clipped for clarity. *B*: afterpotential was depolarized relative to its resting membrane potential with increasing temperature, as shown by the shift in amplitude of the AHP and the ADP in both control and heat-shocked animals. Dotted line indicates original V_m at 25°C. *C*: mean amplitude of the ADP was significantly larger in heat-shocked (open squares) animals compared with control (filled circles) at all temperatures except 25°C. This difference grew larger as temperature increased. *D*: amplitude differences in the ADP were not attributed solely to the increased incidence of ADPs in heat-shocked animals at high temperature. Considering only animals that had a depolarized afterpotential at 40°C, the amplitude of the ADP was significantly larger in heat-shocked animals ($n = 10$) compared with control ($n = 8$) animals. Data are expressed as means ± SE. Significant differences as assessed by a *t*-test ($P < 0.05$) are denoted with asterisks.

The notion that heat shock helps to sustain the role of DCMD in escape behavior is supported by the findings of Barclay and Robertson (2000). In that study, it was shown that heat shock maintains the propensity to execute an escape jump at high temperature, whereas control animals resort to other escape walking behaviors. The authors hypothesized that heat shock prevents a temperature-induced impairment of the higher-order neural processing that initiates the escape behavior. The different levels of DCMD activity in this study between control and heat-shocked animals could explain the different behavioral outcomes found in Barclay and Robertson (2000).

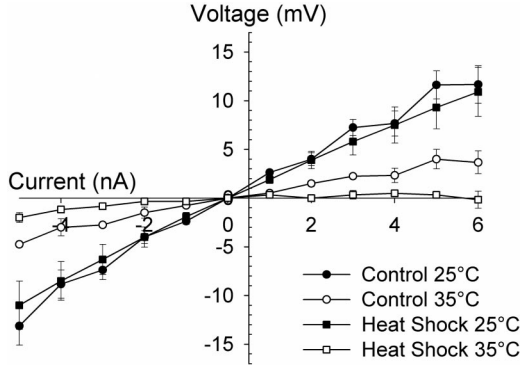

FIG. 8. Voltage vs. current (*V/I*) plots constructed from steady-state voltage responses to steps of injected current. Slope of a linear regression of the *V/I* data were used to estimate input resistance. Voltage responses at 25°C between control (filled circles) and heat-shocked (filled squares) animals were indistinguishable [2-way ANOVA; $F_{(10,87)} = 0.37$, $P = 0.96$], resulting in input resistance values of 2.3 MΩ for control and 2.0 MΩ for heat-shocked animals. Voltage responses at 35°C were reduced in both control (open circles) and heat-shocked (open squares) animals, an effect that was significantly greater after heat shock [2-way ANOVA, $F_{(10,38)} = 10.3$, $P < 0.001$]. Input resistance of DCMD at 35°C was 0.78 MΩ in control and 0.21 MΩ in heat-shocked animals.

Thermosensitivity of individual action potentials is modified by heat shock

Temperature had pronounced effects on the properties of DCMD action potentials. As temperature increased, the resting membrane potential became more hyperpolarized, and action potential amplitude and duration decreased. Together, these effects resulted in a decreased action potential area at high temperatures. These findings are predicted by models of the Goldman–Hodgkin–Katz equation (Johnston and Wu 1995), and have previously been demonstrated in locusts (Burrows 1989; Heitler et al. 1977; Wu et al. 2001; Xu and Robertson 1994).

The thermosensitivity of axonal membrane potential has been shown to vary depending on prior thermal experience (Janssen 1992). The squid giant axon is more hyperpolarized in response to temperature in warm acclimated summer squid compared with squid captured in cooler spring waters (Rosenthal and Bezanilla 2000). Differences in resting membrane potential between animals acclimated to different temperatures can be attributed to altered ion pump activity, particularly increased Na^+/K^+-ATPase activity (Merickel and Kater 1974; Sardella et al. 2004).

In heat-shocked animals, a more hyperpolarized resting membrane potential may help maintain action potential amplitude at high temperature. During high-frequency activity, refractory effects can threaten conduction reliability (Grossman et al. 1979; Zhou and Chiu 2001). Decreased action potential amplitude and conduction failure during high-frequency spiking has been shown in both myelinated (Lüscher et al. 1983; Soleng et al. 2003; Stoney 1990) and unmyelinated axon (Grossman et al. 1979; Smith 1980). Amplitude attenuation can result from the inactivation of voltage-gated sodium current (Rudy 1981). Hyperpolarization reduces the recovery time after Na^+-channel inactivation, which increases the Na^+ con-

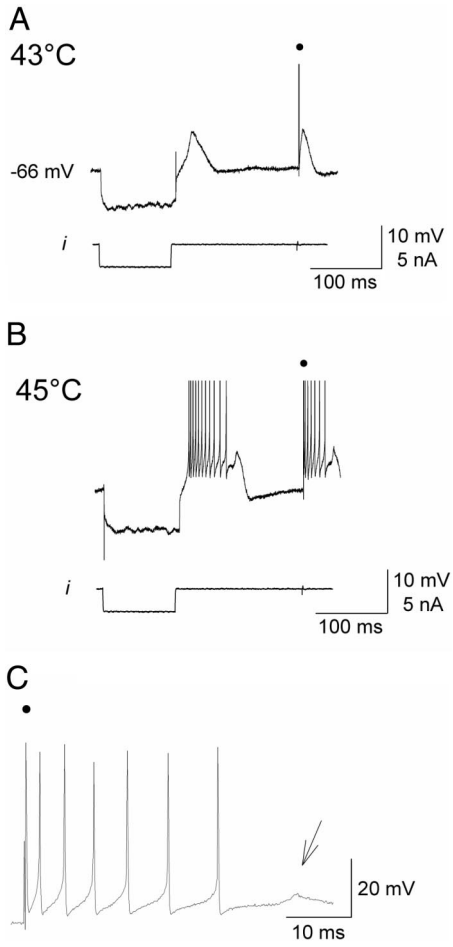

FIG. 9. Increased membrane excitability after heat shock. Periods of increased membrane excitability occurred at temperatures >40°C in heat-shocked animals. Hyperpolarizing current pulses (*i*) were applied through the recording electrode followed 1 s later by a stimulated single action potential (dot on trace denotes the resulting action potential). *A*: induction of a postinhibitory rebound (PIR) after a hyperpolarizing current pulse. *B*: PIR and large ADP events were sufficient to generate a burst of spikes in response to hyperpolarization as well as after electrical stimulation. *C*: stimulation-elicited action potential was followed by a train of spikes with slow activating rising phases. Spike bursts ended with an apparent aborted spike (arrow).

ductance available to participate in the action potential (Jung et al. 1997; Nicholls et al. 1992). Hyperpolarization after heat shock may reduce refractory effects on action potential amplitude during high-frequency activity. Heat shock might also alter the effect of temperature on the kinetics of the Na^+ channels themselves (Bezanilla and Taylor 1978; Schwarz 1986). As resting membrane potential was not manipulated in these experiments, it is not possible to differentiate between these possibilities.

Afterdepolarizations occurred in control animals at high temperature, but they were more prevalent and significantly larger in heat-shocked animals. These findings indicate that a membrane conductance was being altered at high temperatures, revealing an event found infrequently at lower temperatures. An afterpotential event with qualitative similarity to the ADP reported here has been shown previously in locust DCMD (Pearson and Goodman 1981). They suggested that the event resulted from a presynaptic autoinhibition of the DCMD neuron. It is unlikely that the ADP described here is involved in a presynaptic inhibitory mechanism. The recordings from Pearson and Goodman (1981) were made in a ventral branch of DCMD within the metathoracic ganglion, near terminal regions. The recordings from this study were made along the connective in actively conducting axon distant from any such synaptic regions.

Input resistance of DCMD was used as a measure of membrane conductance, and decreased with temperature. This has been shown previously in locust neurons (Xu and Robertson 1994). At 35°C input resistance decreased in heat-shocked animals to near zero. Other stressors, such as hypoxia, produce similarly large decreases in input resistance (Muller and Somjen 2000), which can reach undetectable levels (Snow et al. 1983). The difference in input resistance observed between control and heat-shocked animals may be related to the potentiation of the ADP conductance.

Afterdepolarizations have been described in a variety of neuronal cell types and have been shown to enhance excitability. Many of the recordings from vertebrate neurons are made in the soma or apical dendrites (Kandel and Spenser 1961; Sánchez-Vives and Gallego 1994). In spike-initiating regions the ADP can increase bursting of action potentials (Jensen et al. 1996; White et al. 1989). The ADP has also been shown in the axon, where it contributes to a period of increased excitability that follows the action potential (Bowe et al. 1987; Stys and Waxman 1994). Depolarized afterpotentials have been shown experimentally (Bowe et al. 1987) and in modeling studies (McIntyre et al. 2002) to contribute to a period of decreased threshold after the action potential (supernormal period). In DCMD, a reduced threshold would improve the conduction reliability of high-frequency action potentials in the axon.

Although the nature of the ADP was not explored here, it could be attributable to the potentiation of a depolarizing conductance such as persistent Na^+ (Azouz et al. 1996), T-type calcium (White et al. 1989), or a nonselective cation conductance (Andrew 1987) at high temperature. Alternatively, the ADP could result from a reduced K^+ conductance during the afterpotential, which has been shown to shift the afterpotential toward depolarization (Martinez-Pinna et al. 2000). In each case, the heat shock–induced change would be expected to increase excitability.

In conclusion, we suggest that the observed plasticity in DCMD after heat shock represents an adaptive response to thermal stress. We have shown that at high temperature, heat shock modifies both the activity level and the axonal action potential properties of DCMD. These concurrent effects of heat shock on action potentials may mitigate the refractory effects of high-frequency activity, preserving conduction reliability at high temperature. Future experiments will examine the nature of the heat shock–induced change in the thermosensitivity of voltage-dependent channels in the DCMD axon, and will seek to determine how this plasticity contributes to sustained conduction of high-frequency activity.

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GRANTS

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